Effects of Added Divalent Counterions on the Properties and Behaviors of Microtubule Filaments

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Microtubules are polymeric cytoskeletal filaments that define the shape of eukaryotic cells and are widely involved in intracellular active transport. In the present work, we hypothesized that microtubule mechanics and dynamics may be regulated electrostatically based on their strong polyelectrolyte nature.

We observed that electrostatic screening by low concentrations (1 – 10 mM) of certain divalent ions:, in particular Ba^{2+}:

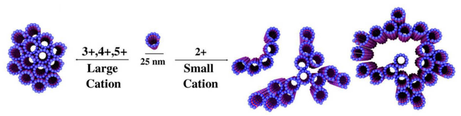
1. **Significantly increases the persistence length (L_p) of microtubules**
2. **Stabilizes microtubules against depolymerization, and**
3. **Passively straightens microtubule trajectories in inverted motility assays**

These changes were attributed to charge neutralization of the C-terminal tubulin tails, as the observed effects were obviated when the tails were cleaved with subtilisin. Moreover, the correlation between the observed changes in stability and mechanical rigidity is consistent that observed for microtubule-associated proteins (MAPs), which also interact with the C-terminal tails. Overall our results establish a novel mechanism by which microtubules dynamics, mechanics, and interaction with molecular motors may be regulated by physiologically relevant concentrations of divalent salts

Introduction

In a solution of polyelectrolytes, the interactions are determined by the collective organization of the counterions. Multivalent counterions are particularly important in biological self-assembly, particularly in mechanisms such as filament bundling (e.g., f-actin) and condensation (e.g., DNA).

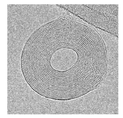
Like-Charge Attraction



Needleman et al. PNAS 2004 16099-103

Bundled filaments: F-actin, Microtubules

Condensation

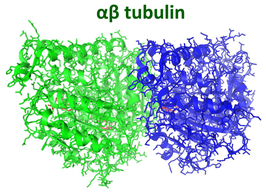


N. V. Hud and K. H. Downing, PNAS 1998 14925–30

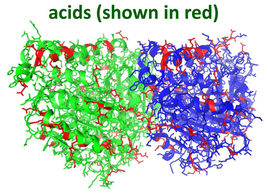
Compact DNA Structures

Microtubules are highly charged structures and may be treated as a biological polyelectrolyte (PE). Therefore, the addition of multivalent ions (at concentrations below the bundling phase boundary) to suspension of microtubules is expected to alter their PE behavior, which in turn may affect their dynamics and interaction with molecular motors.

Crystal Structure αβ tubulin



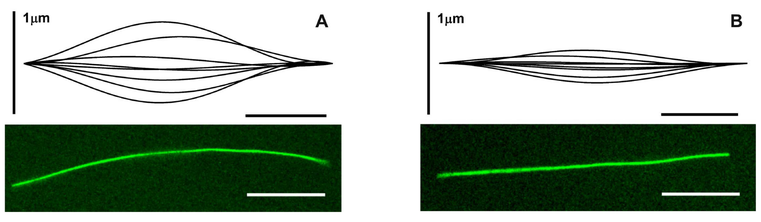
Negatively charged amino acids (shown in red)



Microtubules possess a linear charge density of ~260 e^- μm^{-1} based on the large number of *Asp* and *Glu* amino acids on its surface. In addition, the C-terminal domains (not shown in the crystal structures above) are comprised of an unstructured, highly negatively charged tail (~20 amino acids).

Persistence Length

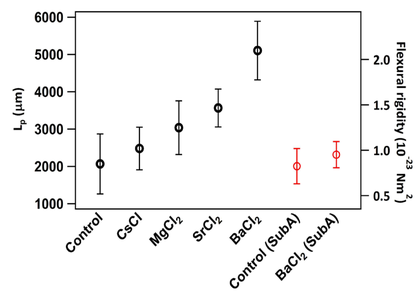
The persistence length (L_p) of microtubule filaments was characterized presence of various mono- and divalent counterions. Qualitative differences are clearly observable in the reconstructed traces and fluorescence photomicrographs of microtubules in the absence (A) and presence of $BaCl_2$ (B).



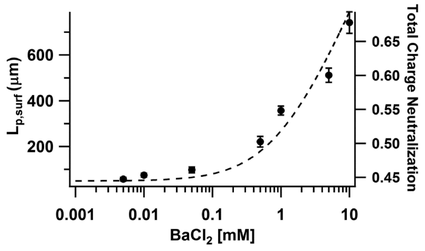
Persistence Length (cont'd)

Significant increases in the L_p of microtubules (right) in the presence of divalent counterions were observed, and correlated strongly with the hydrated size of the counterion ($Mg^{2+} > Sr^{2+} > Ba^{2+}$).

This increase in L_p was not observed when microtubules were treated with subtilisin A (SubA) to remove the C-terminal tubulin tails (upper right, red open circles).



Straighter surface conformations ($L_{p,surf}$) were observed as a function of $BaCl_2$ concentration (right, closed circles). The corresponding charge neutralization fraction of the microtubule surface (dashed line), estimated using Manning's two variable method, correlated well with experimental data for $L_{p,surf}$.

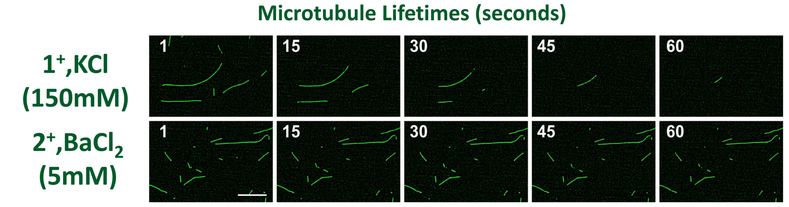


Charge neutralization of the microtubule surface by divalent counterions leads to decreased flexural rigidity, which is, at least in part, related to interaction with the C-terminal tubulin tails.

Dynamic Instability

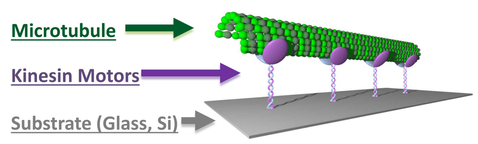
The stability of microtubules (in the absence of Taxol®) was evaluated in the presence of either monovalent (KCl) or divalent ($BaCl_2$) counterions. Control microtubule (KCl) exhibited shortening rates of 30 μm min^{-1} at the minus end and 10 μm min^{-1} at the plus end. In contrast, microtubules with added $BaCl_2$ showed no significant change in length after 7 days.

Microtubule Lifetimes (seconds)

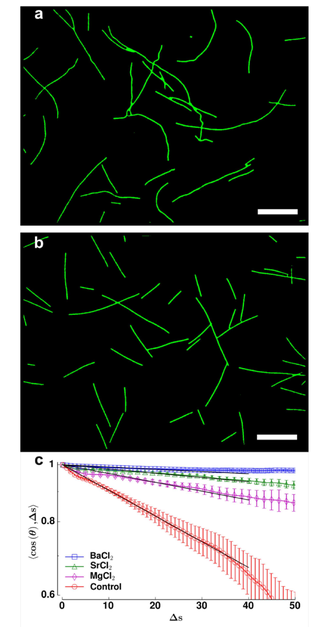


Correlation between increased L_p and stability in the presence of Ba^{2+} is consistent with published observations for microtubule-associated proteins (MAPs) such as Tau. MAPs are known to bind to the C-terminal tails of tubulin, likely through electrostatic interactions.

Passive Steering



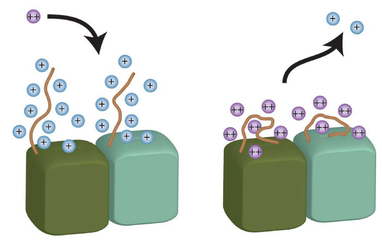
Changes the trajectory of microtubules in kinesin gliding assays reflected the observed increase in L_p in the presence of divalent counterions (right). Here, straighter trajectories were observed when 5 mM Ba^{2+} was added to motility solutions (b, d, f), when compared against controls (a, c, e). This effect could be negated by washing and removing of Ba^{2+} from the flow cell.



Counterion-induced changes in L_p may be used to achieve straighter trajectories, which has applications for passive steering of microtubule transport in hybrid nanosystems.

Conclusion

We conclude that screened coulomb interactions between the microtubule surface and C-terminal tails drive the changes in the rigidity and stability of MTs, as well as affect interactions with kinesin motor proteins, observed in the present study. These data may provide valuable insights as to the role of electrostatics in microtubule dynamics and the interaction between motor proteins and other MAPs with the microtubule surface.



Acknowledgment

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Bouxsein & Bachand, *Biomacromolecules* 15, 3696-3705 (2014)